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Evolution

Archaeal–eubacterial mergers in the origin of Eukarya: Phylogenetic classification of life

(centriole–kinetosome DNA/Protoctista/kingdom classification/symbiogenesis/archaeprotist)

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ABSTRACT **A symbiosis-based phylogeny leads to a consistent, useful classification system for all life. "Kingdoms" and "Domains" are replaced by biological names for the most inclusive taxa: Prokarya (bacteria) and Eukarya (symbiosis-derived nucleated organisms). The earliest Eukarya, anaerobic mastigotes, hypothetically originated from permanent whole-cell fusion between members of Archaea (e.g., *Thermoplasma*-like organisms) and of Eubacteria (e.g., *Spirochaeta*-like organisms). Molecular biology, life-history, and fossil record evidence support the reunification of bacteria as Prokarya while subdividing Eukarya into uniquely defined subtaxa: Protoctista, Animalia, Fungi, and Plantae.**

Bacterial Symbioses Form Cells of Eukarya

Here I detail implications of serial endosymbiotic theory (SET; ref. 1) of origins of nucleated organisms for interpretation of molecular data and classification of all life. Eukaryotes evolved when archaeal (2) (=archaebacterial) and eubacterial cells merged in anaerobic symbiosis. Nucleocytoplasm (from Archaea) acquired swimming motility (from fermenting Eubacteria) becoming mastigotes prior to acquisition of mitochondria or plastids (3, 4). Some mastigotes then incorporated purple Eubacteria (mitochondria precursors) to become oxygen-respiring aerobes from which most protoctists, animals, and fungi evolved. Aerobes in later permanent association with plastid–precursor cyanobacteria became algae—i.e., phototrophic protoctists (5).

Individuality reappears as former symbionts integrate and branches on bifurcating phylogenies anastomose to generate new name-requiring taxa. The necessary and sufficient explanation of simultaneous archaeal and eubacterial protein and nucleic acid sequences (refs. 2, 5–13; Table 1) even in amitochondriate eukaryotes is their origin as genetically integrated bacterial symbionts. The consequences of the SET phylogeny for systematics (evolutionary classification) includes replacement of ad hoc gene transfer hypotheses (10), social-political terms like Kingdom or Domain (2), and other confusion with a consistent taxonomy for identifying, naming, and classifying all life.

Comparable to extant prokaryotic consortia—e.g., *Thiodendron* (15), *Methanobacillus omelianski* (16), and *Daptobacter*-infected chromatia (17)—the earliest symbioses that became eukaryotic cells lacked mitosis and meiosis. Abiding in anoxic environments today are free-living amitochondriate motile heterotrophs, mastigotes, related to animal intestinal symbionts (18, 19). They vary in number of nuclei, organization of chromatin, kinetochores, Golgi–parabasal bodies, rhizoplast nucleus–kinetosome connectors, [9(2)+2] microtubule-based intracellular motility organelles (undulipodia), centriole–kinetosome–mitotic spindles, and meiotic sexuality, suggesting these features evolved in their ancestors by inferable steps (4, 20). rRNA gene sequences (*Trichomonas, Coronympha, Giardia*; ref. 11) confirm these as descendants of anaerobic eukaryotes that evolved prior to the "crown group" (12)—e.g., animals, fungi, or plants.

If eukaryotes began as motility symbioses between Archaea—e.g., *Thermoplasma acidophilum*-like and Eubacteria (*Spirochaeta*-, *Spirosymplokos*-, or *Diplocalyx*-like microbes; ref. 4) where cell–genetic integration led to the nucleus–cytoskeletal system that defines eukaryotes (21)—then an optimal explanation for Table 1's large quantity of otherwise baffling sequence data ensues (Table 2).

Nucleoid membranes are known in at least one prokaryote, *Gemmata obscuriglobus* (24), and membrane hypertrophy is common in invasive associations implying nuclear–endomembrane systems originated as defensive response in bacterial mergers (4). Centriole–kinetosomes are thought derived from attached eubacteria (Fig. 1) such that centriole–kinetosome DNA (c-kDNA; refs. 25 and 26) is their vestige (4, 21).

A great distinction is made between prokaryotic genomes no matter their recombinant source or number per cell (e.g., *Escherichia coli*'s same-genome multiple nucleoids when fission is retarded) and composite genomes derived from genetic integration of symbionts. Archaea and eubacteria, no matter their vast RNA, protein, and metabolic differences, are nevertheless prokaryotes. They lack the system that underlies mendelian genetics (composite heterospecific genomes, intracellular motility, and cell fusion) and therefore are united into one most inclusive taxon: Prokarya. Nucleated organisms, products of symbiont integration of two or more former prokaryotes, are placed in the second taxon: Eukarya.

Protoctista

The Eukarya taxon (equivalent to "Superkingdom" or "Domain") includes amitochondriate mastigotes [Archeozoa (27), Hypochondria (13), here Archaeprotista], organisms derived from symbioses between as few as two prokaryote types. Meiosis and fertilization are known in some: parabasalids (*Barbulanympha*) and pyrsonymphids (*Notila*; ref. 28). Meiotic-sexual cycles did not evolve in all Archaeprotista as Kirby showed for large Calonymphidae trichomonads (29). These amitochondriate multikinetosome mastigotes are multinucleated. They bear numerous akaryomastigonts (four-kinetosome structures that lack their connected nucleus) and karyomastigonts (four-kinetosome organelles attached to a nucleus) peculiar to the family. Akaryomastigont kinesis outpaced karyomastigont kinesis such that the akaryomastigonts outnumber the nuclei in the genera *Calonympha, Stephanonympha*, and *Snyderella* (29). From DNA staining in *Calonympha grassii* akarymastigonts (*) we infer that certain centriole–kinetosomes retained their genes. The nuclear internalization of c-kDNA (i.e., in *Chlamydomonas*; ref. 26) probably oc-

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*Dolan, M., Second European Congress of Protistology, July 24–28, 1995, Clermont-Ferrand, France, p. 42 (abstr.).

Table 1. Prokarya origins of Eukarya components: Eubacterial and archaeal protein contributions to the nucleocytoplasm of eukaryotes

Eubacteria	Archaea
Amino acid metabolism/protein synthesis	
Aspartate aminotransferase	Arginosuccinate synthetase
Glutamine synthetase	Elongation factor 2
Glutamate dehydrogenase	Elongation factor Tu
	Aminoacyl tRNA synthetase (isoleucyl-, leucyl-, valyl-) (8)
	Ubiquitin; proteosomes*
	Modified rRNA[3], 5′ ppp on 5SrRNA*
	Methionine initiation
Fermentation/energy metabolism	
Ferredoxin	Cytoplasmic α-chain, β-chain H^+ vacuolar-type ATPase
Glyceraldehyde-3-phosphate dehydrogenase (9)	Phosphoglycerol kinase
Nucleotide/nucleic acid metabolism	
Formylglycinylmide ribonucleotide synthetase†	Carbamoyl-phosphate synthase‡
Pyrroline-5-carboxylate reductase	Indole-3-glycerol phosphate
	DNA-directed RNA polymerase
	DNA polymerase
	DNA topoisomerase‡
	Histone–DNA binding proteins
	Histidine pathway‡
	Fibrillamina of nucleolus
Most membranes	
Ester-linked lipids, phospholipids	Amino acid–Na^+ cotransport protein
	Isoprenoid membrane lipids (ethers)
Stress protein	
hsp70 (DnaK)	hsp60 chaperonin, cocytes (TCP-1)
Vitamin and storage metabolism	
	Dihydrofolate reductase
	Glycogen or cytoplasmic "floridean" starch
Carbohydrate metabolism	
	Dolichol pathway of glycosylation
	N-linked carbohydrates

This table was constructed in collaboration with D. G. Searcy and R. S. Gupta. Details of proteins and organisms are given in refs. 6 and 7. Nucleocytoplasm is exclusive of mitochondria, plastids, and any other subsequently acquired genomes.
*Similarities for cocytes only.
†B. Golding, personal communication.
‡Ref. 14 or A. Lazcano, personal communication.

Table 2. Hypothetical source of eukaryotic features

Spirochaeta sp. (or related eubacterium)	*Thermoplasma* sp. (or related archae)
Undulipodium: undulating swimming motility	Acid, heat resistance
Centriole–kinetosomes (centrosomes, centroplasts)	Membrane ATPases and lipids, H^+-ATPase
Spindle tubules, axonemes	Chromatin with nucleosomal organization, histones, lamins
Eubacterial genes, proteins*	Archaebacterial polymerases and protein-synthetic apparatus; other archaebacterial enzymes*
O_2 hypersensitivity	O_2 microaerophilia
Retraction†	Pleiomorphism (absence of cell wall)
H_2 production‡	Sulfur respiration§
Resistant propagule formation†	Cytoskeletal fibers¶

Emergent after fusion and integration: amitochondriate mastigotes (Phylum Archaeprotista of Table 4), nuclear envelope with 8-fold symmetrical pores, mitosis, Ca^{2+}-sensitive physiologies, actin- and myosin-based phagocytosis, fluid (reversibly fusing) membranes, motility-based morphogenesis, programmed cell hybrid formation.
*See Table 1 (6, 7).
†*Spirosymplokos deltaeiberi* provides an example of a spirochete preadaptation of undulipodial withdrawal into the cell and perhaps even resistant propagule formation (22).
‡Fermentation product.
§*Thermoplasma* grows anaerobically if in contact with elemental sulfur particles as electron acceptors.
¶These structures reported in mycoplasms (23); their presence in *Thermoplasma* requires investigation.

curred concomitant with the evolution of mitotic/meiotic cycles in ancestral protoctists (4).

The protoctists include archaeprotists, stramenopiles (heterokonts), alveolates (ciliates, apicomplexa, and dinomastigotes) and all other eukaryotes not animal, plant, or fungus (Table 3). Since all except archaeprotists have mitochondria (with or without plastids) they evolved by symbioses from more than two former prokaryotes. The earliest fossil protoctists are hard-walled 2.0 billion-year-old spherical microfossils, acritarchs (35). The large Eukarya are younger: animal fossils first occur in sediments dated 600–520 million years, whereas the earliest fungi and plants appear 450–400 million years ago (36).

The number of integrated former symbionts comprising many protoctists is unknown [e.g., fibrillar bodies of planktic foraminifera may have originated by bacterial symbioses (37); "Nebenkorper" (*Paramoeba eilhardi*) may be former bacteria (38); nucleomorphs of cryptomonads may involve two or three (nucleocytoplasm/plastid/mitochondria) genomes per host (13)]. Most protoctist taxa are trigenomic at least since they are mitochondriate aerobes (nucleocytoplasm/centriole-kinetosome/mitochondria). These include malarial parasites, ciliates, and amoebae, whereas those of at least four genomes (nucleocytoplasm/centriole–kinetosome/mitochondria/plastids) include euglenids, diatoms, chrysophytes, coccolithophorids, and brown and red algae (Table 3). Protoctists in which the cells are large (30–500 μm in greatest dimension—e.g., heliozoa, foraminifera) harbor many DNA-positive organelles among which genetic relations are unknown. The number of former symbionts (integrated heterologous genomes) is expected to increase as the genetics and molecular biology of the protoctists become better understood.

Previous analyses that recognize the importance of symbiosis in cell evolution (Schwemmler, ref. 39; co-founder of "Endocytobiology," ref. 40; and Taylor, ref. 1, inventor of monad-dyad-polymonad terminology) are used here. Eukarya includes, in addition to protoctists, three molecularly homogeneous lineages: Animalia, Plantae, and Fungi. The minimum number of integrated former symbionts of Animalia is three (nucleocytoplasm/centriole–kinetosome/mitochondrion) or four including deDuve's peroxisomes (from Gram-positive eubacteria; ref. 41). If Scannerini and Bonfante-Fasolo (42) properly interpret "BLOs" (bacteria-like organelles), then Fungi evolved from four or five originally independent prokaryotes: nucleocytoplasm/mitochondrion/peroxisome/BLO and/or centriole-kinetosome. More dramatically, if Atsatt's (43) and Pirozynski's (44) hypothesis of plant origins from green algae and fungal genome is confirmed, then the integrated genome numbers in Plantae may exceed seven (nucleocytoplasm/centriole–kinetosome/mitochondrion/peroxisome-glyoxysome/fungal nucleocytoplasm/fungal centriole–kinetosome equivalent/peroxisome–glyoxisome/fungal mitochondrion).

Interaction of former bacterial symbionts of Eukarya may underlie cyclical development distinguishing protoctists and "crown-taxa" life cycles. Identification of originally indepen-

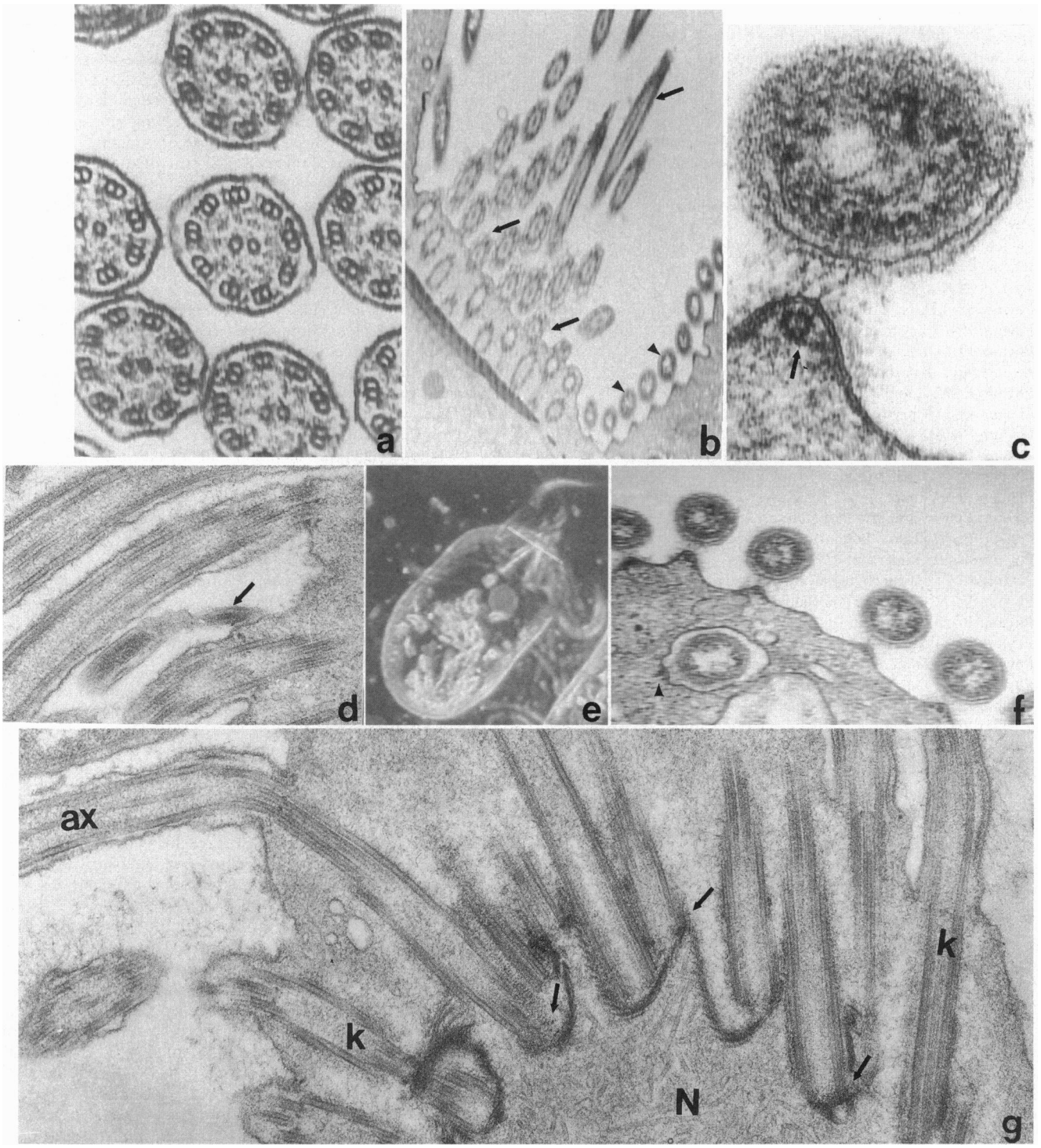

FIG. 1. Kinetosomes vs. bacterial attachments in Archaeprotist mastigotes from termites. (*a*) Axonemes (ax) and kinetosomes (k) of undulipodia of *Staurojoenina assimilis* in *Incisitermes minor.* (*b*) Undulipodia (arrows), symbiotic bacteria (arrowheads). (*c*) A host microtubule underlies host membrane at each attached bacterium (arrow). (*d*) Undulipodia at cell surface, spirochete (arrow). (*e*) Live *Staurojoenina*; bar at position of electron micrographs. (*f*) Distribution of surface and intracellular symbiotic bacteria, attachment fiber development at arrowhead. (*g*) Kinetosome–nuclear (N) associations (arrow) at surface of related parabasalid from *Reticulitermes hesperus.* Anoxic environment, no mitochondria. EM by D. Chase.

dent genomes in nucleated individuals is not required for taxonomy but becomes a major goal of molecular biology.

Ploidy, Gamonts, and Gametes

In protoctists, reproduction (increase in number of offspring) and sexuality (the formation of a new individual by fusion of genetic material from more than a single parent) are entirely separable. Life cycle, symbioses, and structural diversity of these organisms so transcend botanical–zoological experience that plant–animal terminology distorts their description. To compensate, Grell (38) introduced protoctist life-cycle terms indispensable for Eukarya phylogenetic analysis.

Gametes (cells or nuclei of complementary genders) require merger for further development, minimally karyogamy (fusion of nuclei) and usually cytogamy (fusion of cytoplasm). Ga-

Table 3. Taxon ("Kingdom") Protoctista

Etymology
Gr. *Protos*-, very first; *Ktista*, established beings
History of use of term: see legend
Definition: see legend
Phyla list with examples
Phylum Pr-1 Archaeprotista* (*Giardia, Microspora*)
Phylum Pr-2 Rhizopoda (amoebae, cellular slime molds)
Phylum Pr-3 Granuloreticulosa* (inc. foraminifera)
Phylum Pr-4 Xenophyophora (deep sea enigmas)
Phylum Pr-5 Myxomycota* (plasmodial slime molds, *Physarum*)
Phylum Pr-6 Dinomastigota* (*dinoflagellates Gonyaulux*)
Phylum Pr-7 Ciliophora* (*Paramecium*)*
Phylum Pr-8 Apicomplexa* (*Plasmodium*)
Phylum Pr-9 Haptomonads (prymnesiophytes coccolithophorids)
Phylum Pr-10 Cryptomonads (*Copromonas*)
Phylum Pr-11 Discomitochondrios (euglenids, kinetoplastids, amoebomastigotes)
Phylum Pr-12 Zoomastigina (*Proteromonas*)
Phylum Pr-13 Chrysomonads (*Ochromonas*)
Phylum Pr-14 Xanthophyta (*Vaucheria*)
Phylum Pr-15 Eustigmatophyta (eyespot algae)
Phylum Pr-16 Bacillariophyta* (diatoms, *Navicula*)
Phylum Pr-17 Phaeophyta* (kelp, brown algae, *Fucus*)
Phylum Pr-18 Labyrinthulids/Thraustochytrids (slime nets)
Phylum Pr-19 Plasmodiophorids (plant parasites)
Phylum Pr-20 Oomycota* (*Phytopthora*)
Phylum Pr-21 Hyphochytriomycota (zoosporic mycelia)
Phylum Pr-22 Haplosporidia (haplosporosome formers)
Phylum Pr-23 Paramyxea (nesting cell-forming parasites)
Phylum Pr-24 Myxozoa (*Actinomyxa*)
Phylum Pr-25 Rhodophyta* (red seaweeds, *Gracilaria*)
Phylum Pr-26 Gamophyta* (Conjugaphyta, desmids)
Phylum Pr-27 Chlorophyta* (*Chlamydomonas*)
Phylum Pr-28 Actinopoda* (heliozoa, radiolaria)
Phylum Pr-29 Chytridiomycota* (*Blastocladiella Neocallimastix*)

History of use of term. John Hogg's four kingdoms, illustrated in color, are Animal, Vegetable, Mineral, and Primogenium. He placed Protoctista, all organisms neither animal nor plant, in kingdom Primogenium (30). Copeland (31) added phylum "Inophyta" (the akontous zygo-, asco-, and basidiomycetous fungi) to Protoctista making four Kingdoms of life: Monera (Haeckel's bacteria), Plantae, Animalia, and Hogg's Protoctista. After Whittaker raised fungi to kingdom status (32, 33) Schwartz and I reunited eukaryotic microorganisms with their multicellular relatives as protoctists modifying Whittaker's five kingdom scheme (34).

Definition. Protoctista include eukaryotic microorganisms and their larger descendants exclusive of three large taxa: (Animalia) diploids that develop from blastulas, (Plantae) haplo-diploid embryo-formers that undergo sporogenic meiosis, and Fungi (= Mychota) akontous conjugating mycelial spore-forming haploid or dikaryotic osmotrophs. Protoctista, products of symbiogenesis, evolved by permanent cell fusion as associations between differing bacteria. Fused DNA–protein systems led to chromatin [125-Å (diameter) unit fibrils], intracellular motility including mitosis, and cell fusions (syngamy, karyogamy) cyclically relieved by meiosis (28). Membranes contain steroids and polyunsaturated fatty acids perhaps related to periodic cell fusions. Many form cellulosic, chitinous, polysaccharide, proteinaceous, mineralized (carbonate, siliceous) walls outside the membranes. Included are all algae, microscopic phagotrophs and other heterotrophs (mistakenly called "Protozoa" which are not animals), water molds, other lineages, each with unicellular and derived multicellular members. Aquatic in all habitats below 70°C, they range in size from <1 μm to >100 m. Although the term Protoctista encompasses the entire group, some use Protista (which generally refers to smaller protoctists) as synonym for this higher taxon.

*All except Pr-1 have mitochondria. Meiotic-fertilization mendelian genetic systems well-established in some species.

monts, who at maturity are capable of becoming or producing gametes, are either haploid or diploid organisms. (Gamont and/or gamete differentiation and fusion are often stress induced.) Haploid nuclei or cells, representing the haploid phase of the haplo-diploid life cycle, are not necessarily gametes: many grow by mitosis and become adults capable not of sex but of reproduction. A mature organism, regardless of ploidy, capable of reproduction but not gamete production is an agamont. The diplophase begins by fully viable intraspecific nuclear fusion. Complementary genders (mates, conjugants, or their representatives) proceed by cytogamy to dikaryosis. The dikaryon, fused cells or multicellular organisms with at least two nuclei each from a different source, later forms a zygote by karyogamy establishing the diploid nucleus. The diplophase terminates with meiosis (reductive cell division). Many Eukarya secondarily lost biparental sex of mendelian genetic systems. Grell's analysis applies to meiotically sexual relatives and predecessors: most protoctists, fungi, and (because they develop from sexually produced embryos) all animals and plants.

Table 4. Taxonomic summary

Prokarya = Prokaryotae
Monohomogenomic prokaryotic cells, chromonemal genetic organization ultrastructurally visible as nucleoids. Cell-to-cell transfer of genophores—i.e., of the chromoneme (large replicons) and of plasmids (and other small replicons). No cytoplasmic fusion. Flagellar (rotary motor) motility.
Bacteria: (Monera, Prokaryotae, Procaryotae)* Bacterial cell organization, monohomogenomic
Archaea (methanogens, thermoacidophiles, halophiles, and probably some Gram-positive bacteria)
Eubacteria (Gram-negative and most other bacteria, extraordinary range of metabolic modes)
Eukarya = Eukaryotae
Polyheterogenomic† eukaryotic cells, products of integrated bacterial symbioses. Chromosomal genetic organization in nuclei intracellular motility (actin–myosin), microtubule organizing centers (tubulin–dynein–kinesin), reversible nuclear and cell fusion. Meiosis/fertilization cycles underlie mendelian genetic systems.
Protoctista (30)
Mitotic organisms, motile by undulipodia; meiosis/fertilization cycles absent or variable‡
Fungi
Hyphal fusion, zygotic meiosis to form resistant propagules (spores), lack undulipodia.
Plantae
Maternally retained embryo formed from fusion of mitotically produced gamete nuclei, sporogenic meiosis.
Animalia
Blastula formed after fusion of anisogametes (fertilization of egg by sperm), gametic meiosis.

*Groups equivalent to former "Domains" or "Superkingdoms" ***italicized boldtype***, present "subkingdoms" are *italicized*, equivalent to former "Kingdoms" underlined; except for Eukarya (which is still equivalent to a "Superkingdom").
†Poly=many, hetero=other, genomic=sum of all genes in an individual.
‡See Table 3 and ref. 46 for fossil record.

Criteria for Classification

Molecular sequence changes, by-products of metabolism and of chance, may persist unhoned by natural selection. Although natural selection refers to the differential production of RNA molecules, genes, organisms, communities, or any reproducing system with high heritability, in nature not molecules but whole organisms are selected. Natural selection acts throughout the life history of all organisms in specific habitats at given times. Therefore, whole-organism biology (including all genetics and metabolism that determines life cycle) not just molecular sequence must undergird phylogenetic classification. Further-

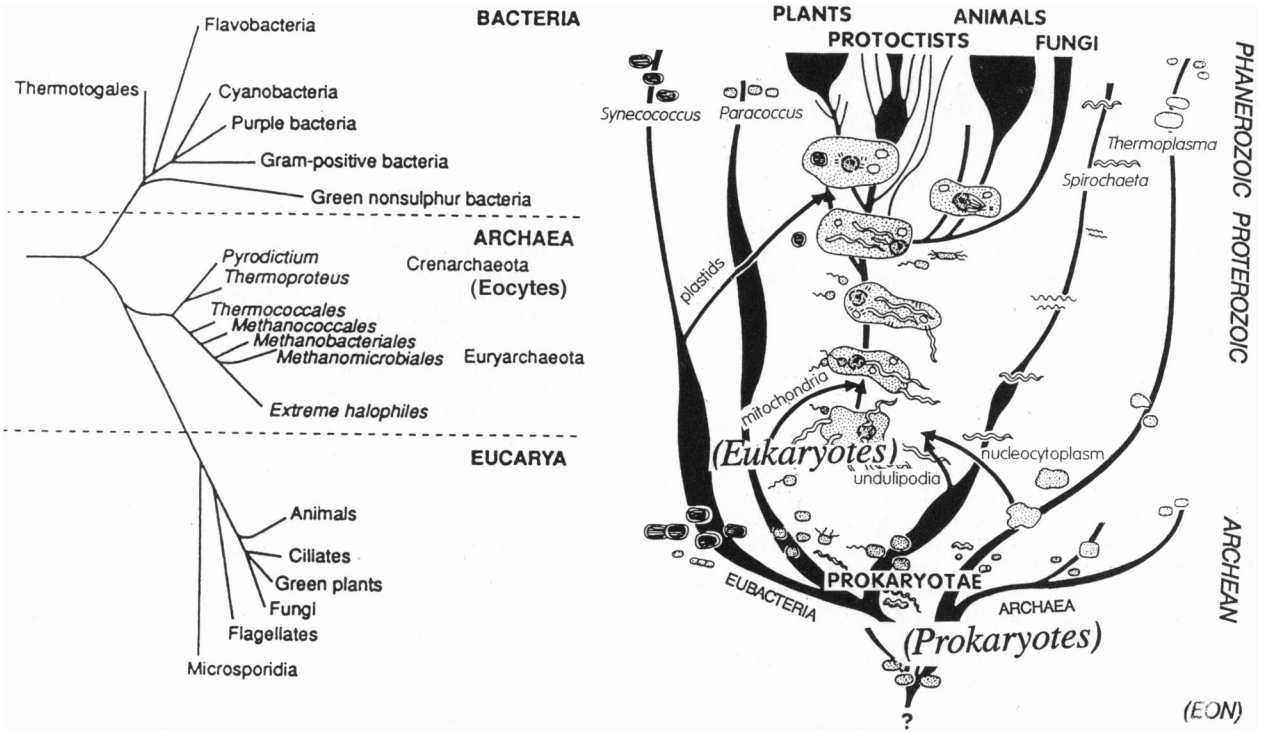

FIG. 2. The three-domain scheme (2) (*Left*) compared to the evolution of five most inclusive taxa detailed here against the geological time scale. Details in ref. 4 (*Right*).

more, internally consistent evolutionary-taxonomic schemes are essential for those dealing with life (naturalists, educators, physicians) and time (paleontologists, stratigraphers). Molecular biologists and chemists, who extract informative molecules from life, can only provide crucial independent methods to confirm or disprove evolutionary scenarios.

Major innovations evolving in prokaryotes (e.g., metabolic pathways and nutritional modes: hetero-, chemolitho- or photoautotrophy, cell wall composition, locomotion, sensory systems) are useless to distinguish Eukarya. Neither can multicellularity delimit taxa because all lineages including the oldest known life (Prokarya as 3500 my old fossil bacteria) and the later-appearing Eukarya (2000 my) have multicellular descendants.

The three remaining Eukarya taxa in order of appearance in the record are as follows:

Animals. Animals as mature diploid gamonts (adults) meiotically form unequal (aniso-) gametes (undulipodiated sperm and egg cells) that fertilize (cytogamy). A short dikaryotic stage, followed by karyogamy, forms the zygote from which the blastula develops. Generally the blastula gastrulates to lay down the asymmetrical axes (mouth, anus) of the growing embryo ("Kingdom" Animalia).

Plants. Plants are haplo-diploid organisms that produce haploid gamonts mitotically from spores. Haplophase organisms ("gametophytes") form unequal (aniso-) gametes by mitosis within multicellular sexual organs (antheridia with undulipodiated sperm, archegonia, gametangia). Cytogamy of complementary genders is followed by karyogamy (single or multiple gamete nuclei) to form zygote nuclei. The resulting embryos (agamonts) of the diplophase are retained by the female haploid gamont. The mature diplophase agamont (the multicellular sporophyte) produces haploid spores by meiosis. Reinitiating the haplophase, meiosis in plants is sporogenic. Haploid spores develop into male (antheridia- or pollen-forming) or female (archegonial- or embryo sac-forming) haploid gamonts. In most plants, haploid gamonts mitotically develop both male and female multicellular sexual organs on the same individual ("Kingdom" Plantae).

Fungi. Fungi germinate from resistant propagules (fungal spores), haploid uni- or multinucleate cells. Most fungi are haploid agamonts capable of rapid reproduction by asexual airborne spores (conidiae, chlamydospores, etc.). Either mitotically (asexually) or meiotically produced spores tend to be more resistant to heat, desiccation, starvation, etc., than the growing phase of the same organism. Spores germinate into agamonts (single cells of yeasts or hyphae of molds, morels, mushrooms) that represent either the agamont or the haploid gamont. The haplophase, especially in basidiomycotes, does not necessarily terminate with conjugation (isogamontous fusion) because cytogamy may greatly precede karygamy to form stable dikarya. Many more than two genders (genetically compatible conjugants) may be present in a single species. The dikaryotic fungal haplophase terminates with karyogamy; after fusion the diploid nuclei quickly undergo two meiotic divisions (zygotic meiosis). The products of meiosis are not gametes nor are undulipodia present at any stage in the life history. Rather, sexual fusions lead to spores (ascospores, basidiospores, etc.) capable of germination. ("K." Mychota)

This phylogenetic classification summarized (Table 4) is compared with Woese's (2) three-kingdom scheme (Fig. 2). Whether prokaryotic or eukaryotic, cells contain at least five types of nucleic acid (DNA, mRNA, tRNA, small and large subunit rRNA) and at least 500 different proteins (45), no single one of which is usable alone as an adequate phylogenetic marker for history of the lineage. Yet it is precisely because they may each represent different aspects of evolutionary history of the organisms from which they are extracted that molecular sequences permit tests of phylogenetic inferences and classification schemes.

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